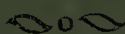


SOME NEW PHYSIOLOGICALLY  
ACTIVE DERIVATIVES OF  
CHOLINE

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*(Reprinted from 'The Biochemical Journal,' Vol. VIII, No. 4, 1914)*



*From*

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## XLII. SOME NEW PHYSIOLOGICALLY ACTIVE DERIVATIVES OF CHOLINE.

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(*Received July 10th, 1914.*)

A short time ago I described [Ewins, 1914, 1] the isolation from extract of ergot, of a new active principle which was identified as acetyl-choline. The action of this base was shown by Dale [1914] to be closely related to that of muscarine, the active principle of the fungus *Amanita muscaria*, to which Harnack [1875] assigned the formula  $C_5H_{15}O_3N$ , and suggested that it was an oxidation product of choline. "Synthetic" muscarine (pseudo-muscarine), originally obtained by Schmiedeberg and Harnack [1877], and considered by them to be identical with muscarine, but shown by later observers to differ from natural muscarine in physiological action, I have recently shown to be the choline ester of nitrous acid [Ewins, 1914, 2]. The constitution of the natural muscarine, however, still remained (and remains) unsolved, but from the fact that acetyl-choline and pseudo-muscarine, both of which are choline esters, very closely resemble the natural base in physiological action, we were led to suppose that muscarine might also be an ester of choline. Some support was given to this idea when we found that the nitric acid ester of choline, originally prepared by Schmidt and Wagner [1904], still more closely resembled muscarine in its action. It was found, however, that the action of extracts of *Amanita muscaria* was not appreciably lessened by boiling with dilute acid or alkali, so that it seems highly improbable that the natural base is a choline ester.

Since the amount of *Amanita muscaria*<sup>1</sup> at our disposal was too small to admit of isolation of the natural base in a state of purity, or in any quantity,

<sup>1</sup> For an extract of *Amanita muscaria* we are indebted to Dr O. Rosenheim, and for a quantity of dried material to Prof. W. Wiechowski, to both of whom we wish to render our best thanks.

I prepared a number of the simpler choline derivatives which might possibly give a clue to the constitution of the natural base. So far the investigation has not led to the identification of muscarine itself, but certain of the derivatives prepared were of some considerable interest, the ethyl ether of choline  $\text{OH} \cdot \text{N}(\text{CH}_3)_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{O} \cdot \text{C}_2\text{H}_5$  in particular being very closely related indeed in its action to that of the natural muscarine. The bases were in all cases physiologically examined by Dr H. H. Dale, whose results will be published shortly.

The following is a table of the substances examined :

(1)	Acetyl-choline	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{OCCH}_3.$
(2)	<i>Formyl-choline</i>	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{OCH}.$ $\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{CHCH}_3.$
(3)	Lactyl-choline	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{CO}.$
(4)	Choline nitrous acid ester			$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{NO}.$
(5)	Choline nitric acid ester			$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{NO}_2.$
(6)	Choline methyl ether	..		$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{CH}_3.$
(7)	Choline ethyl ether	..		$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{C}_2\text{H}_5.$
(8)	<i>Choline propyl ether</i>	..		$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{C}_3\text{H}_7.$
(9)	<i>Choline ether</i>	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{O} \cdot \text{CH}_2 \text{CH}_2 \text{N}(\text{CH}_3)_3 \text{OH}.$
(10)	Trimethyl-β-bromo-ethyl-ammonium bromide			$\text{BrN}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{Br}.$
(11)	Trimethyl-β-chloro-ethyl-ammonium chloride			$\text{ClN}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{Cl}.$
(12)	<i>Trimethyl-β-cyano-ethyl-ammonium hydroxide</i>			$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{CN}.$
(13)	<i>Trimethyl-β-amino-ethyl-ammonium hydroxide</i>			$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{CH}_2 \text{NH}_2.$
(14)	<i>Dimethyl-oxazolium hydroxide</i>	..	..	$(\text{OH}) \text{N}(\text{CH}_3)_2 - \text{CH}_2$ $\quad \quad \quad   \quad \quad \quad  $ $\quad \quad \quad \text{CH}_2 \cdot \text{CH}_2 \cdot \text{O}$
(15)	<i>Formocholine methyl ether</i>	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{OCH}_3.$
(16)	<i>Formocholine ethyl ether</i>	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{OC}_2\text{H}_5.$
(17)	<i>Formocholine propyl ether</i>	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{OC}_3\text{H}_7.$
(18)	<i>Formocholine butyl ether</i>	..	..	$\text{OH} \cdot \text{N}(\text{CH}_3)_3 \text{CH}_2 \text{OC}_4\text{H}_9.$

All of these bases showed considerable physiological activity, those approaching most nearly to muscarine being choline ethyl ether, choline nitric acid ester, and trimethyl-β-amino-ethyl ammonium hydroxide. The curare-like action of all of these is, however, more pronounced than in the case of natural muscarine.

The ethers of formocholine were found to possess an action closely resembling that of the corresponding choline ethers, but were quantitatively much less powerful. Formocholine propyl ether was found to be the most active of the series whereas the isomeric choline ethyl ether was the most active of the choline ethers.

The compounds printed in italics in the above table are new substances. Their preparation and properties are described below. The remaining bases were obtained by the methods previously employed by other workers, notably Schmidt and Wagner [1904] and Nothnagel [1894].



## EXPERIMENTAL.

(a) *Choline derivatives.*

*Formyl-choline*,  $\text{OH} \cdot \text{N}(\text{CH}_3)_3\text{CH}_2\text{CH}_2\text{O} \cdot \text{OCH}$ . The base was obtained by boiling a solution of choline chloride in formic acid (D 1.22) under reflux for three hours. The excess of formic acid was removed by distillation *in vacuo*. The residue was dissolved in absolute alcohol and converted into the platinichloride by precipitation with an alcoholic solution of platinic chloride. The precipitate was filtered off and crystallised from hot water in which it is readily soluble. On standing, the crystalline *platinichloride* was obtained in orange-red octahedra. M.p.  $255-256^\circ$ .

0.1111; 0.0840  $\text{CO}_2$ ; 0.0450  $\text{H}_2\text{O}$ . C=20.6; H=4.5.

0.1087; 0.0313 Pt. Pt=28.8.

Calculated for  $(\text{N}(\text{CH}_3)_3\text{CH}_2\text{CH}_2\text{O} \cdot \text{OCH})_2\text{PtCl}_6$ . C=21.4; H=4.2; Pt=29.0.

The *aurichloride* crystallised from hot water in thin golden yellow hexagonal plates. M.p.  $175^\circ$ .

0.1168; 0.0488 Au; Au=41.8.

Calculated for  $\text{N}(\text{CH}_3)_3\text{CH}_2\text{CH}_2\text{O} \cdot \text{OCH} \cdot \text{AuCl}_4$ ; Au=41.8.

*Choline propyl ether*,  $\text{OH} \cdot \text{N}(\text{CH}_3)_3\text{CH}_2\text{CH}_2\text{O} \cdot \text{C}_3\text{H}_7$ . This was obtained by acting on  $\beta$ -iodo-ethyl propyl ether [Karvonen, 1909] with alcoholic trimethylamine solution at the ordinary temperature. The crystalline iodide of choline propyl ether separated on standing, and was filtered off, converted into the chloride in the usual manner and then into the *platini-chloride*, which was obtained crystalline from hot aqueous solution.

Orange red prisms. M.p.  $246^\circ$ .

0.1205; 0.1197  $\text{CO}_2$ ; 0.0626  $\text{H}_2\text{O}$ ; C=27.1; H=5.75.

0.0922; 0.0262 Pt; Pt=28.4.

Calculated for  $(\text{C}_8\text{H}_{20}\text{ON})_2\text{PtCl}_6$ . C=27.4; H=5.7; Pt=27.9.

*Choline ether*,  $\text{OH} \cdot \text{N}(\text{CH}_3)_3\text{CH}_2\text{CH}_2\text{O} \cdot \text{CH}_2\text{CH}_2(\text{CH}_3)_3\text{N} \cdot \text{OH}$ .  $\beta\beta$ -Diiodo-ethyl ether [Sand, 1901] was treated with a slight excess of alcoholic trimethylamine solution at the ordinary temperature. On standing, the crystalline choline ether iodide rapidly separated, and was filtered off after about twenty-four hours.

The *iodide* crystallises from 95 per cent. alcohol in thin rectangular plates melting at  $275^\circ$ . The salt is very readily soluble in water, but sparingly soluble in absolute alcohol.

0.0736; 0.0781 Ag I; I=57.3.

Calculated for  $\text{C}_{10}\text{H}_{26}\text{ON}_2\text{I}_2$ ; I=57.2.

(b) *Formo-choline derivatives.*

Formo-choline itself was obtained by Schmidt and Litterscheid [1904] who were, however, unable to obtain either acetyl, benzoyl, or lactyl derivatives. Attempts by the writer to prepare the nitric ester of the base by methods similar to those employed for the preparation of the corresponding choline ester proved unsuccessful. It was found, however, that the ethers of formo-choline could readily be obtained by the action of trimethylamine on the corresponding iodomethyl ethers.

*Formo-choline methyl ether*,  $\text{OH} \cdot \text{N}(\text{CH}_3)_3\text{CH}_2\text{O} \cdot \text{CH}_3$ . Iodomethyl ethyl ether  $\text{I} \cdot \text{CH}_2\text{O} \cdot \text{CH}_3$  was prepared according to the method employed by Henry [1893]. For purification it was washed with a very little ice water, dissolved in ether and dried over  $\text{CaCl}_2$ . The ether was removed and the residue distilled *in vacuo*. It boiled constantly at  $39^\circ$  under 20 mm. pressure.

To the ethereal solution of the iodo-ether was added alcoholic trimethylamine, the mixture being kept cool by immersion in ice. A crystalline product consisting of a mixture of formo-choline methyl ether iodide, and trimethylamine iodide, separated immediately. The mixture was suspended in cold absolute alcohol. The bulk of the trimethylamine iodide remained undissolved and was filtered off. The filtrate was evaporated to dryness, and the residue crystallised from a little alcohol by addition of ether. Formo-choline ether iodide was thus obtained as white glistening hygroscopic plates, melting at  $84^\circ$ .

0.1421 ; 0.1428 Ag I ;  $\text{I} = 54.5$ .  
Calculated for  $\text{CH}_3\text{OCH}_2\text{N}(\text{CH}_3)_3\text{I}$  ;  $\text{I} = 54.98$ .

The *platinichloride* crystallised from water in columnar aggregates of orange-red rhombic prisms, melting with decomposition at  $234^\circ$ . The salt was anhydrous and sparingly soluble in cold water, but readily soluble in hot water.

0.1028 ; 0.0322 Pt ;  $\text{Pt} = 31.3$ .  
Calculated for  $(\text{C}_5\text{H}_{14}\text{ON})_2\text{PtCl}_6$  ;  $\text{Pt} = 31.6$ .

The *aurichloride* is moderately soluble in hot water, sparingly in cold. It crystallises in golden yellow needles melting at  $135$ – $136^\circ$ .

0.1212 ; 0.0536 Au ;  $\text{Au} = 44.2$ .  
Calculated for  $\text{C}_5\text{H}_{14}\text{ON} \cdot \text{AuCl}_4$  ;  $\text{Au} = 44.4$ .

*Formo-choline ethyl ether*,  $\text{OH} \cdot \text{N}(\text{CH}_3)_3\text{CH}_2\text{O} \cdot \text{C}_2\text{H}_5$ . Iodo-methyl ethyl ether,  $\text{I} \cdot \text{CH}_2\text{O} \cdot \text{C}_2\text{H}_5$ , was prepared by a method analogous to that employed by Henry to obtain the corresponding methyl ether. On distillation *in vacuo*



a liquid was obtained which boiled constantly at a temperature of  $44^{\circ}$  at 30 mm. The product, however, on analysis was found to be still somewhat impure, but owing to the highly reactive nature of the liquid it was not further purified, but treated at once with trimethylamine, and the product worked up in the same manner as described for the corresponding methyl ether.

*Formo-choline ethyl ether iodide* was thus obtained as thin lustrous deliquescent plates, melting at  $94^{\circ}$ .

0.1100 ; 0.1066 AgI ;  $I=52.3$ .

Calculated for  $C_2H_5OCH_2N(CH_3)_3I$  ;  $I=51.8$ .

The *platinichloride* crystallised from hot water in large regular crystals orange-red in colour and melting at  $241-242^{\circ}$ . It is moderately soluble even in cold water, but quite insoluble in alcohol.

0.1052 ; 0.0321 Pt ;  $Pt=30.5$ .

Calculated for  $(C_6H_{16}ON)_2PtCl_6$  ;  $Pt=30.3$ .

The *aurichloride* crystallised from water in golden yellow leaflets melting at  $138-139^{\circ}$ .

0.1426 ; 0.0618 Au ;  $Au=43.3$ .

Calculated for  $C_6H_{16}ONAuCl_4$  ;  $Au=43.1$ .

*Formo-choline propyl ether*,  $OH.N(CH_3)_3CH_2O.C_3H_7$ . *Iodomethyl propyl ether*  $I.CH_2O.C_3H_7$  (b.p.  $61-62^{\circ}$  at 28 mm.) was obtained by the method employed in the previous preparations, treated with trimethylamine, and the product worked up as before.

*Formo-choline propyl ether iodide* was thus obtained crystalline from alcohol by addition of ether as long fine needles melting at  $108^{\circ}$ . The salt was not very deliquescent.

0.1201 ; 0.1112 AgI ;  $I=50.0$ .

Calculated for  $C_3H_7OCH_2N(CH_3)_3I$  ;  $I=49.0$ .

The *platinichloride* was obtained crystalline from hot water, in which the salt is very easily soluble. It is insoluble in alcohol and very little soluble in cold water ; long thin orange-red needles melting at  $236-237^{\circ}$ .

0.1165 ; 0.0342 Pt ;  $Pt=29.35$ .

Calculated for  $(C_7H_{18}ON)_2PtCl_6$  ;  $Pt=29.0$ .

The *aurichloride* is only moderately soluble even in hot water. It crystallises in golden yellow leaflets melting at  $114^{\circ}$ .

0.1094 ; 0.0460 Au ;  $Au=42.0$ .

Calculated for  $C_7H_{18}ONAuCl_4$  ;  $Au=41.6$ .

*Formo-choline butyl ether*,  $OH.N(CH_3)_3CH_2O.C_4H_9$ , was prepared by the action of trimethylamine on iodomethyl butyl ether  $I.CH_2O.C_4H_9$  (b.p.

78–80° at 20 mm.). The *iodide* crystallised in glistening colourless plates melting at 98°.

0.1812; 0.1610 AgI; I=48.0.  
Calculated for  $C_8H_{20}ONI$ ; I=46.5.

The *platinichloride* crystallises from hot water as orange-red octahedra melting at 243–244°. It is insoluble in alcohol, sparingly soluble in cold, but fairly readily soluble in hot water.

0.0992; 0.0276 Pt; Pt=27.7.  
Calculated for  $(C_8H_{20}ON)_2PtCl_6$ ; Pt=27.8.

The *aurichloride* is little soluble in cold water, moderately so in hot. It crystallises in golden yellow leaflets melting at 81°.

0.1110; 0.0450 Au; Au=40.5.  
Calculated for  $C_8H_{20}ONAuCl_4$ ; Au=40.6.

#### REFERENCES.

- Dale (1914), *J. Physiol.* **48**, *Proc.* iii.  
Ewins (1914, 1), *Biochem. J.* **8**, 44.  
— (1914, 2), *Biochem. J.* **8**, 209.  
Harnack (1875), *Archiv exp. Path. Pharm.* **4**, 168.  
Henry (1893), *Ber.* **26**, *Ref.* 934.  
— (1898), *Bull. Acad. Roy. Belg.* [3], **35**, 360.  
Karvonen (1909), *Ber.* **42**, 687.  
Nothnagel (1894), *Arch. Pharm.* **232**, 268.  
Sand (1901), *Ber.* **34**, 2906.  
Schmidt and Wagner (1904), *Annalen*, **337**, 37.  
Schmidt and Litterscheid (1904), *Annalen*, **337**, 37.  
Schmiedeberg and Harnack (1877), *Arch. exp. Path. Pharm.* **6**, 101.





